NUCLEIC ACID AND AMINO ACID SEQUENCES OF INFECTIOUS SALMON ANAEMIA VIRUS AND THEIR USES AS VACCINES

The present invention relates to a fish vaccine. More specifically the invention relates to a vaccine to protect salmon against infectious salmon anaemia virus.

Infectious salmon anaemia virus (ISAV) causes mortality of farmed Atlantic salmon. Typically aquaculture revenue is reduced by over 30%. Accordingly, there is a need for an effective vaccine against ISAV.

It is an object of the present invention to provide a vaccine to protect against ISAV.

According to the present invention there is provided a composition containing at least one nucleic acid sequence and/or at least one amino acid sequence, or a synthetically prepared analogue thereof or a substantially homologous sequence, wherein the composition is derived from or based upon infectious salmon anaemia virus and wherein at least one of said nucleotide and/or amino acid sequences does not cause salmon anaemia and is capable of being used as or to prepare a vaccine to ISAV.

A substantially homologous nucleic acid sequence is a sequence which can be transcribed and/or translated to provide an amino acid sequence which is substantially

homologous to at least a part of a surface antigen present on ISAV.

Preferably the substantially homologous amino acid is at least 70% homologous with a part of a surface antigen of ISAV which is capable of inducing an immune response.

More preferably the substantially homologous amino acid sequence is at least 80% homologous with a part of a surface antigen of ISAV and can induce an immune response.

Most preferably the substantially homologous amino acid sequence is at least 90% homologous with a part of a surface antigen of ISAV and can induce an immune response.

Suitably the amino acid sequence is chosen from the group comprising Sequences ID numbers 2, 4, 6, 7, 8 or 10 as herein described.

Alternatively the amino acid sequence may comprise at least one fragment of Sequence ID numbers 2, 4, 6, 7, 8 or 10.
Alternatively said amino acid sequence may be truncated from an amino acid sequence of Sequences ID numbers 2, 4, 6, 7, 8 or 10 as herein described, which can induce an immune response.

Preferably the substantially homologous nucleotide sequence is at least 60% homologous with a part of a nucleic acid sequence of a surface antigen of ISAV and

the translation product thereof is capable of inducing an immune response.

Preferably the substantially homologous nucleotide sequence encodes at least 70% homologous with a part of a nucleic acid sequence of a surface antigen of ISAV, the translation product of which is capable of inducing an immune response.

More preferably the substantially homologous nucleotide sequence encodes at least 80% homologous with a part of a nucleic acid sequence of a surface antigen of ISAV, the translation product of which is capable of inducing an immune response.

Most preferably the substantially homologous nucleotide sequence is at least 90% homologous to a part of a nucleic acid sequence of a surface antigen of ISAV, the translation product of which is capable of inducing an immune response.

Suitably the nucleotide sequences are chosen from the group comprising Sequence ID numbers 1, 3, 5 or 9 as herein described.

Alternatively, the invention provides for fragments of the sequences described in Sequence ID numbers 1, 3, 5 and 9 as herein described and wherein translation products of said fragments result in the induction of an immune response.

Additionally, the sequences may comprise a truncated form of the sequences given as 1, 3, 5 and 9.

The nucleotide sequence may be incorporated in a plasmid.

The nucleotide sequence may be incorporated in a suitable expression vector.

A further aspect of the present invention provides for the use of a sequence chosen from the group consisting of Sequence ID numbers 1 to 10, as described in the present invention in the preparation of a vaccine and/or therapeutic medicament for the protection of fish from infection with Infectious Salmon Anaemia virus.

Typical nucleic acid sequences are ISA2cd (previously referred to as p1.38), ISA1mta (previously referred to as p8.17), ISA3mx (previously referred to as p6.28) and ISA4ha.

Preferably the peptide sequences are transcribed and translated from either one, two or all of the nucleic acid sequences; ISA2cd, ISA1mta, ISA3mx or ISA4ha and are incorporated into a vaccination strategy aimed at inducing an immune response to a surface antigen of ISAV and thus infectious salmon anaemia virus itself.

The invention provides the use of nucleic acid sequences or peptide sequences as defined herein in the preparation of a vaccine for the protection of fish against ISAV.

The invention further provides a vaccine to protect fish against ISAV wherein the vaccine includes nucleic acid or peptide sequences as defined herein.

Characterisation of the Novel Sequences of the Invention

The accompanying figures describe the invention in more detail, wherein;

Figure 1 is the nucleotide sequence of ISA2cd,

Figure 2 is the amino acid sequence which is obtained from translation of the ISA2cd nucleic acid sequence listed in Figure 1,

Figure 3 is the nucleotide sequence of ISA1mta,

Figure 4 is the amino acid sequence which is obtained following transcription of the nucleic acid sequence listed in Figure 3,

Figure 5 is the exact nucleotide sequence of ISA3mx,

Figure 6a is the amino acid sequence (M1) which is translated from the unspliced nucleic acid sequence of ISA3mx shown in Figure 5,

Figure 6b is the amino acid sequence (M2) which is translated from the spliced nucleic acid sequence of ISA3mx shown in Figure 5, and

Figure 6c is the amino acid sequence (M3) which is translated from the unspliced nucleic acid sequence of ISA3mx as shown in Figure 5.

In addition, information detailing the specific molecular weight (MW) and theoretical isoelectric focusing points (pI) is given at the foot of the respective amino acid sequence listings.

The nucleotide and amino acid sequences shown in the figures are further represented in the accompanying Patent-In generated sequence listings wherein;

Sequence ID number 1 is the nucleotide sequence of ISA2cd, as shown on figure 1,

Sequence ID Number 2 is the amino acid sequence of the ISA2cd, as shown in figure 2,

Sequence ID number 3 is the nucleotide sequence of ISA1mta, as shown on figure 3,

Sequence ID number 4 is the amino acid sequence of ISA1mta, as shown on figure 4,

Sequence ID number 5 is the nucleotide sequence of ISA3mx, as shown on figure 5,

Sequence ID number 6 is the predicted amino acid sequence of unspliced product of ISA3mx, as shown in figure 6a,

Sequence ID number 7 is the predicted amino acid sequence of spliced ISA3mx, as shown in figure 6b,

Sequence ID number 8 is the predicted amino acid sequence of spliced ISA3mx, as shown in figure 6c,

Sequence ID number 9 is the nucleotide sequence of ISA4ha, as previously shown in figure 7, and

Sequence ID number 10 is the amino acid sequence of ISA4ha, as previously shown in figure 8.

The genetic sequences shown for ISA1mta and ISA2cd and the unspliced and spliced genetic sequences for ISA3mx have been derived from cloned cDNA wherein the cDNA clones were derived from infectious salmon anaemia virus (ISAV) genomic material. The cloned material was sequenced from the 5' end and the 3' end insertion sites using overlapping amplicons to produce a contig.

Veracity of the contig was confirmed by Reverse Transcriptase Polymerase Chain Reaction amplification (RT-PCR) of appropriate sized amplicons from ISAV infected salmon tissue and tissue cultures. Such amplicons were however obtained from uninfected control material, indicating that the genetic material was of ISAV origin.

The open reading frames (ORFs) were completed by rapid amplification of cDNA ends (RACE) from the incomplete sequence from virus-infected tissue culture. Corrections were made for the *in vivo* transcribed mRNA

that were not apparent from the originally cloned cDNAs.

The ORF from ISA2cd does not have any significant homology at the nucleotide or amino acid sequence with previous submissions to databases accessible by BLAST. However, proteins with similar molecular weights (Mw) and isoelectric points (pI) include 14 viral proteins in the Swiss-Prot database such as Hemagglutinin-Neuraminidase.

The ORF from ISA1mta is also without any significant homology to previously characterised proteins submitted to the BLAST searchable databases. However it is of interest that it has molecular weight and isoelectric point characteristics (68-69 kDa and pI 8.2) that are nearly identical to one of the most predominant viral proteins identified by two dimensional electrophoresis. The protein appears to be integrally associated with the membranes of the ISAV infected tissue cultures. If the ORF yields such a protein it would be considered valuable in any vaccination strategy to reduce the level of ISAV infection in any salmonoid species.

Further, in the sequences shown for ISA3mx, the unspliced ORF (the basis for predicted amino acid sequence M1) does not have any significant homology at the nucleotide or amino acid sequence level with the previous submission to databases accessible by BLAST. However, proteins with similar molecular weights and isoelectric focusing points include several viral coat and envelope proteins listed in the Swiss-Prot database. Both the predicted M1 and M2 proteins

(obtained from ORF's following splicing of the nucleotide sequence) are predicted to be membrane associated proteins and if the ORFs encoded by ISA3mx yield such proteins it would be considered valuable in any vaccination strategy to reduce the level of ISAV infection in any salomonid species.

The predicted protein translation of M3 (shown in figure 6c and accompanying sequence listing) shows homology to a paromyxovirus fusion protein associated with the cell membrane and thought to be involved in cell adhesion. In view of this exhibited homology, M3 is potentially valuable in any vaccination strategy aimed at reducing the level of ISAV infection in any salmonid species.

The further sequence relating to ISA4ha nucleotide sequence was obtained by means of the following procedure. The ISA4ha protein was detected by polyclonal antibodies following hybridisation. The protein is found to occur in two alternative forms. These two alternative forms are of different sizes, and can be seen where the proteins are cultured on different cell lines, for example shc and chse.

As these two alternate forms were both detectable by antibody and varied in size depending on how it was grown, the protein is potentially a good candidate for virulence.

The protein was isolated and sequenced, resulting in a 24 amino acid fragment being produced. When this sequence was submitted, to BLAST searchable databases,

it showed similarities to sequences of British and Norwegian strains of ISAV.

Subsequently, primers were designed based on the amino acid sequence obtained, along with reference to the sequences known for the similar British and Norwegian strains.

The primers were then subsequently used in polymerase chain reaction to amplify the relevant DNA fragment, which was subsequently sequenced and translated into amino acid coding.

The open reading frame listings obtained in the present invention, have particular commercial value for the following reasons:

1. There is sufficient reason to believe that the nucleotide corresponding amino acid sequences are of ISAV origin. Therefore, their incorporation into nucleic acid vaccines may have an impact on the reduction of mortality of farmed Atlantic salmon caused by ISAV which as previously stated, can typically reduce aquaculture revenues by over 30%.
2. Characterisation of the gene product will lead to the identification of key elements in the pathogenesis of infection and to the design of more accurate diagnostic tests which will also aid in epidemiological studies documenting the dissemination of different strains of the disease.

The nucleotide sequences ISA1mta, ISA2cd, ISA3mx, ISA4ha and associated derivatives thereof when translated into protein sequences being composed of either identical or equivalent amino acids, should induce a response by the hosts immune system. This principle can be further expanded to use these proteins in diagnostics tests and vaccination procedures.